Expression of E-cadherin and β-Catenin in Human Non-Small Cell Lung Cancer and the Clinical Significance

Shinichiro Kase, Kenji Sugio, Tatsuro Okamoto, Tokuiro Yano, and Keizo Sugimachi
Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan

ABSTRACT
E-cadherin, a calcium-dependent cell-cell adhesion molecule, plays a key role in the maintenance of tissue integrity. The function of this molecule is partly mediated by α/β-γ-catenin. Loss or dysfunction of E-cadherin is associated with an invasive phenotype. We analyzed the expression of E-cadherin and β-catenin in human lung cancer to determine the relationship to clinicopathological factors and prognosis. E-cadherin and β-catenin expressions were evaluated in 331 lung cancer tissues in a immunohistochemical analysis. Reduced E-cadherin expression was evident in 138 (42%), and reduced β-catenin expression was noted in 122 (37%). Reduced E-cadherin expression significantly correlated with lymph nodes metastasis (P = 0.0199). E-cadherin expression significantly correlated with increasing histological differentiation (P = 0.0403). Although reduced E-cadherin did not correlate with the prognosis (P = 0.0652), reduced β-catenin expression did significantly correlate with a poor prognosis (P = 0.0001). When both were reduced, there was a significant unfavorable prognosis compared with either the reduced expression (P = 0.0493) and preserved expression (P = 0.0003). Multivariate analysis showed a significantly lower survival rate for patients with reduced β-catenin (P < 0.0001). We interpret these data to mean that dysfunction of the cell-cell adhesion molecule has a role in the progression of lung cancer and that analysis of E-cadherin and β-catenin expression can provide clinically important evidence on which to base treatment.

INTRODUCTION
Lung cancer, a common cause of death in Japan, is frequently highly invasive or metastatic at the time of initial diagnosis. Even without nodal metastasis, the 5-year survival is 50–70% (1–5), and death follows metastatic spread. Cells detach from the primary tumor, invade vessels, circulate through the entire body, and then reattach at the metastatic site (6). More reliable prognostic factors are needed at the time of resection.

E-cadherin is the prime mediator of intercellular adhesion in epithelial cells. This transmembrane glycoprotein, localized mainly in adherens junctions, mediates by extracellular domain cell-cell adhesion through calcium-dependent, homotypic interactions. The carboxy cytoplasmic domain of this molecule is associated with a group of undercoat proteins, termed catenins (α/β-γ-catenin; Ref. 7). E-cadherin binds directly to β-catenin, and α-catenin links the bound E-cadherin complex to the actin cytoskeleton. This binding is essential for formation of stable cell-cell adhesion and is partly regulated by β-catenin. β-Catenin has homology to human plakoglobin, a component of desmosomal plaques and adherens junctions (8), and to the product of Drosophila segment polarity gene armadillo (9, 10). β-Catenin may differ from cadherin-mediated cell adhesion, because β-catenin participates in a signaling pathway that specifies embryonic patterning (11). In addition, it was reported that the APC (3) tumor suppressor gene product forms a complex with β-catenin, and disruption of this complex is a crucial step in colorectal carcinogenesis (12, 13). As a consequence, mutation in either APC (3) or β-catenin leads to the accumulation of cytoplasmic β-catenin, which binds to T-cell factor and lymphoid enhancer factor transcription factors (14, 15).

E-cadherin and β-catenin expression is reduced in tumor progression and metastasis and the prognosis is poor in cases of the esophagus (16–20), stomach (21–24), colon (25), liver (26, 27), pancreas (28), and urinary bladder (29–32). There is little documentation regarding the immunohistochemical expression of these molecules in lung cancer, and reduced E-cadherin expression correlates with differentiation, lymph node metastasis, an advanced clinical stage, and a poor prognosis (33–36). β-Catenin expression was reported by Reitera et al. (37), but the significance was not fully determined. We evaluated the expression of E-cadherin and β-catenin in cases of human lung cancer, and the relationship between expression and clinical features was given attention.

PATIENTS AND METHODS
Patients and Tissue Specimens. Between 1990 and 1998, 331 Japanese patients with NSCLC were referred to Kyushu University Hospital for surgery. None of these patients had undergone chemotherapy or radiotherapy prior to the surgery. Complete resection was done for 279 patients, and incomplete resection was done for 52 patients. Incomplete resection means macroscopic evidence of the tumor or metastatic lymph node...
nodes left behind, microscopic evidence of the tumor on the resected stump, or clinical evidence of a distant metastasis. There were 209 men and 123 women, with ages ranging from 26 to 87 years (mean, 65.82 years). Histologically, 227 were adenocarcinomas and 104 were squamous cell carcinomas. One hundred and seventy-four had stage I disease, 41 had stage II disease, 64 had stage IIIA disease, 40 had stage IIIB disease, and 12 had stage IV disease. One hundred and thirty-two had T1 disease, 133 T2 disease, 27 had T4 disease, and 39 had T4 disease. Two hundred and six had N0 disease, 58 had N1 disease, 133 T2 disease, 27 had T3 disease, and 39 had T4 disease. Two hundred and six had N0 disease, 58 had N1 disease, 64 had stage IIIA disease, 40 had stage IIIB disease, and 104 were squamous cell carcinomas. One hundred and seventy-four had stage I disease, 41 had stage II disease, 64 had stage IIIA disease, 40 had stage IIIB disease, and 12 had stage IV disease. Total follow-up ranged from 8 to 105 months. Surgically resected tumor specimens were fixed with 10% formalin and embedded in paraffin, and 3-μm-thick sections were prepared.

**Immunohistochemistry.** Sections were deparaffinized in xylene three times for 5 min each and then placed in a graded series of ethanol (100, 90, 80, and 70%). To enhance antigen retrieval, sections were pretreated in an autoclave at 121°C for 5 min in 0.01 M citrate buffer (pH 6.0) and cooled to room temperature, and to quench the endogenous peroxidase activity, the sections were processed to 0.5% H2O2 in methanol for 30 min and then rinsed in PBS three times for 5 min each. The sections were incubated with 10% rabbit normal serum for 20 min at room temperature. After this blocking, sections were incubated overnight at 4°C with primary antibodies at 1:200 dilution, one was a mouse monoclonal antibody against human E-cadherin (C20820) and the other a human monoclonal antibody against human E-cadherin (C19220) antibody purchased from Transduction Laboratories (Lexington, KY). The sections were rinsed three times with PBS for 5 min each and sequentially incubated with biotinylated secondary antibodies for 15 min at room temperature, rinsed three times for 5 min each with PBS and streptavidin-biotin-peroxidase for 5 min at room temperature, and then rinsed three times with PBS for 5 min. The peroxidase reaction was visualized by making use of a solution of 3,3′-diaminobenzidine tetrahydrochloride-supplemented 0.2% hydrogen peroxide in PBS. The sections were then lightly counterstained with hematoxylin. Paraffin-embedded tissues from normal colon epithelium of the homogeneous immunophenotype for the studied antigens were included as positive controls. These colon epithelium tissues were obtained from patients undergoing surgery for colon cancer.

**Immunohistochemical Assessment.** The staining was localized mainly on membranes of the tumor cells. The rate of staining of the tumor cells was estimated as a percentage of >500 tumor cells in five fields selected at random (×400) and scored in one of the following categories: (a) preserved expression ≥70% of tumor cells were stained; and (b) reduced expression: <70% of tumor cells were stained.

Distribution of the ratio of stained cells showed bipolarity, and we separated these into two groups, at the level of 70%. Necrotic areas were not taken into consideration. Heterogeneous staining was classified into reduced expression when <70% of the tumor cells were stained. All tumor slides were examined at random by two investigators who were unaware of the clinical data.

**Western Blotting.** In addition to immunohistochemical analysis, tissues stored at −110°C until analysis were evaluated by Western blotting. We rapidly homogenized the tissue in 2× sample buffer [125 mM Tris (pH 6.8), 4% SDS, 10% glycerol, and 4% β-mercaptoethanol], centrifuged the homogenate, diluted an aliquot of the sample at least 10-fold to be used for bicinchoninic acid protein, using assay kits purchased from Pierce (Rockford, IL). We then added 2× sample buffer to the samples to 5 mg protein/ml and applied 10 μl (50 μg of protein) of tissue homogenates to each well of 7.5% polyacrylamide and 0.75-mm-thick gels. Proteins were electrophoretically transferred to nitrocellulose membrane. To saturate nonspecific protein binding sites, we incubated the membrane in TBST [20 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.05% Tween 20] + 1% blot-qualified BSA for 30 min. To bind the primary antibody, we replaced the blocking solution with TBST containing a dilution of the primary antibody (1:2500 for E-cadherin antibody and 1:500 for β-catenin antibody), followed by incubation for 60 min, with gentle agitation. To remove any unbound antibody, we washed the membrane in TBST three times for 5 min each time. Next, the membrane was transferred to TBST containing anti-IgG alkaline phosphatase conjugated and incubated for 30 min. The membrane was washed in TBST three times for 5 min each to remove any unbound secondary antibody. The membrane was then placed into color development solution and incubated until the bands reached the desired intensity, and then the reaction was halted by washing the membrane for two minutes in deionized water.

**Statistical Analysis.** Correlations between antigen expression and clinicopathological factors were evaluated using χ2 Fisher’s exact test. Data preserved from the date of complete surgical resection to death of the patient were analyzed using the Kaplan-Meier method, and the differences were evaluated using the log-rank test. The prognostic significance of E-cadherin and β-catenin expression concerning other pathological variables was assessed using multivariate Cox proportional hazard’s analysis. P < 0.05 was considered to have statistical significance.

**RESULTS**

**Expression of E-cadherin and β-Catenin.** The expression of E-cadherin was localized mainly on membranes of the tumor cells (Fig. 1A), but in some cases it was localized in the cytoplasm. There were some cases of heterogeneous staining, all of which accounted for <70% of the stained cells. The expression of β-catenin was also localized mainly on membranes of the tumor cells (Fig. 1B), but staining was localized in the cytoplasm in 10% of the cases. Similar to E-cadherin, there were some cases of heterogeneous staining, all of which also accounted for <70% of the stained cells. With Western blot analysis, M, 120,000 of protein was strongly expressed with use of an E-cadherin antibody in cases of preserved expression of E-cadherin, whereas no protein was expressed in the case of reduced expression of E-cadherin (Fig. 2). Similarly, Western blot analysis showed that M, 92,000 of protein was expressed by β-catenin antibody, preserved expression of β-catenin strongly expressed β-catenin, whereas the reduced expression of β-catenin weakly expressed β-catenin (Fig. 2). Therefore, the preserved expression of E-cadherin and β-catenin in this immunohistochemical analysis expressed E-cadherin and β-catenin by Western blotting analysis, and the reduced expression of E-cadherin and β-catenin by immunohistochemical analysis did
not express or only weakly expressed E-cadherin and β-catenin by Western blotting analysis.

Clinicopathological Factors According to the Expression of E-cadherin and β-Catenin. In 331 tumors immunostained by E-cadherin antibody, 193 (58%) showed the preserved expression and 138 tumors (42%) showed the reduced expression. There were no significant correlations between E-cadherin expression and histology, pT, and lymphatic invasion.

Immunohistochemical expression of E-cadherin significantly correlated with histological differentiation ($P = 0.0403$; Table 1). In 206 cases without lymph node metastasis, 76 (36.5%) showed the reduced expression, and 130 (63.5%) showed the preserved expression. In 125 with lymph node metastasis, 62 (49.6%) showed the reduced expression, and 63 (50.4%) showed the preserved expression. There was a significant inverse correlation between E-cadherin expression and lymph node metastasis.
Expression of E-cadherin and β-catenin showed the preserved expression. No significant correlation between E-cadherin expression and vascular invasion (P = 0.3321; Table 2).

In all of the same 331 tumors immunostained with β-catenin antibody, 209 (63%) showed the preserved expression, and 122 (37%) tumors showed the reduced expression. There were no significant correlations between β-catenin expression and histology, differentiation, pT, pN, vascular invasion, or lymphatic invasion (Table 2).

Correlation between E-cadherin and β-Catenin Expression. Results of E-cadherin and β-catenin coexpression in 331 tumors are shown in Table 3. One hundred and ninety-three with the preserved expression of E-cadherin showed 141 (73%) of the preserved expression of β-catenin, and 52 (27%) showed the reduced expression of β-catenin, whereas 138 of the reduced expression of E-cadherin showed 68 (49%) of the preserved expression of β-catenin, and 70 (51%) showed the reduced expression of β-catenin. The correlation between E-cadherin and β-catenin expression was statistically significant (P < 0.0001; Table 3).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinicopathological characteristics of 331 lung cancer patients according to the expression of E-cadherin</th>
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</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td></td>
<td>Squamous cell carcinoma</td>
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<tr>
<td>Tumor differentiation</td>
<td>Well</td>
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<td></td>
<td>Moderate</td>
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<td>Poorly</td>
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<tr>
<td>T factor</td>
<td>pT1</td>
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<td></td>
<td>pT2</td>
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<td></td>
<td>pT3</td>
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<td></td>
<td>pT4</td>
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<tr>
<td>N factor</td>
<td>pN(−)</td>
</tr>
<tr>
<td></td>
<td>pN(+)</td>
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<tr>
<td>Vascular invasion</td>
<td>(+)</td>
</tr>
<tr>
<td></td>
<td>(−)</td>
</tr>
<tr>
<td>Lymphatic invasion</td>
<td>(+)</td>
</tr>
<tr>
<td></td>
<td>(−)</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>pN(−)</td>
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<tr>
<td></td>
<td>pN(+)</td>
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<tr>
<td>Squamous cell carcinoma</td>
<td>pN(−)</td>
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<tr>
<td></td>
<td>pN(+)</td>
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node metastasis (P = 0.0199; Table 1) and a significant inverse correlation between E-cadherin expression and vascular invasion (P = 0.0202; Table 1).

In cases of adenocarcinoma, 146 cases without lymph node metastasis, 61 (41%) showed the reduced expression and 85 (59%) the preserved expression, whereas in 81 with lymph node metastasis, 38 (47%) showed the reduced expression and 43 (53%) showed the preserved expression. No significant correlation was observed between E-cadherin expression and lymph node metastasis. In cases of squamous cell carcinoma, in 60 cases without lymph node metastasis, 15 (25%) showed the reduced expression, and 45 (75%) showed the preserved expression, whereas in 44 with lymph node metastasis, 24 (54.5%) showed the reduced expression and 20 (45.5%) showed the preserved expression. There was a significant inverse correlation between E-cadherin expression and lymph node metastasis in cases of squamous cell carcinoma (P = 0.0021; Table 1).

Correlation between E-cadherin and β-Catenin Expression. Results of E-cadherin and β-catenin coexpression in 331 tumors are shown in Table 3. One hundred and ninety-three with the preserved expression of E-cadherin showed 141 (73%) of the preserved expression of β-catenin, and 52 (27%) showed the reduced expression of β-catenin, whereas 138 of the reduced expression of E-cadherin showed 68 (49%) of the preserved expression of β-catenin, and 70 (51%) showed the reduced expression of β-catenin. The correlation between E-cadherin and β-catenin expression was statistically significant (P < 0.0001; Table 3).
Expression of E-cadherin and β-Catenin and Survival Time. Results of E-cadherin and β-catenin expression were analyzed with regard to survival time. With regard to the expression of E-cadherin, the lung cancer-related 5-year survival rates were 66.2% in the preserved expression and 56.3% in the reduced expression. Although a significant difference was not observed between the two groups, patients with the reduced expression tended to have a poorer prognosis than did those with the preserved expression ($P = 0.0652$; Fig. 3). With regard to the expression of β-catenin, the lung cancer-related 5-year survival rate was 69.1% in case of the preserved expression and 46.0% for the reduced expression, and patients with the reduced expression of β-catenin had a significantly poorer prognosis than did those with the preserved expression of β-catenin ($P = 0.0001$; Fig. 4). Next, survival rate was analyzed, based on the histology. With regard to E-cadherin for both adenocarcinoma and squamous cell carcinoma, correlations were nil. In relation to β-catenin in cases of adenocarcinoma, the 5-year survival rate was 46% for the reduced expression and 82% for the preserved expression. The reduced expression of β-catenin in adenocarcinoma significantly correlated with a poorer prognosis than did the preserved expression ($P = 0.0001$; Fig. 4). Next, survival rate was analyzed, based on the histology. With regard to E-cadherin for both adenocarcinoma and squamous cell carcinoma, correlations were nil. In relation to β-catenin in cases of adenocarcinoma, the 5-year survival rate was 46% for the reduced expression and 82% for the preserved expression. The reduced expression of β-catenin in adenocarcinoma significantly correlated with a poorer prognosis than did the preserved expression ($P = 0.0001$; data not shown). In cases of squamous cell carcinoma, there was no significant correlation regarding survival time between preserved and reduced expression of β-catenin. The 5-year survival rate was 72.4% in cases of the preserved expression for both E-cadherin and β-catenin and 58.2% in cases of the reduced expression for either E-cadherin or β-catenin, and 45.8% in cases of the reduced expression for both E-cadherin and β-catenin. Cases of the reduced expression of both E-cadherin and β-catenin showed a significant unfavorable prognosis compared with cases of the reduced expression of either E-cadherin or β-catenin ($P = 0.0493$; Fig. 5) and compared with cases of the preserved expression of both E-cadherin and β-catenin ($P = 0.0003$; Fig. 5).

To further evaluate the expression of E-cadherin and β-catenin as prognostic factors, a multivariate Cox regression analysis was carried out. In an analysis of 331 patients, which included tumor stage, lymph node metastasis, distant metastasis, histological type, differentiation, expression of E-cadherin, and expression of β-catenin, reduced β-catenin expression showed an independent prognostic factor ($P = 0.0007$) as did lymph node metastasis ($P < 0.0001$; Table 4).
DISCUSSION

In this study, we evaluated immunohistochemically the expression of E-cadherin and β-catenin in formalin-fixed, paraffin-embedded tissue specimens of lung cancer tissue, and we analyzed clinicopathological findings, vascular and lymphatic invasion, as related to survival time. One hundred and thirty-eight of 331 (42%) of tumors showed reduced expression of E-cadherin, and 122 of 331 (37%) showed reduced expression of β-catenin. The reduced expression of E-cadherin correlated with lymph node metastasis, and the reduced expression of β-catenin correlated with a poor prognosis.

Previous studies showed that the rate of reduced expression of E-cadherin of lung carcinomas was 44–81% (33, 34, 36, 38). Sulzer et al. (33) stated that when clear staining was present in <50% of the tumor cell population, the result was defined as negative or weakly positive. They did not mention the rate of negative or weakly positive cases. According to Bohn et al. (34), the E-cadherin expression level was classified as reduced when fluorescence intensity was markedly less than that of adjacent normal epithelium and/or 90–5% of the tumor cells were stained and as absent when staining was not distinguishable from background or <5% of the tumor cells were stained, the result being that 35% were the reduced type and 18% were the absent type (39), findings similar to ours. Bongiorno et al. (17) discussed the preserved type, disorganized type, and reduced type as a classification. Twelve (23%) showed the preserved type, 11 (21%) showed the reduced type, and 29 (56%) showed the disorganized type of E-cadherin expression, with the total reduced and disorganized types being 40 (77%). This result was much higher than ours, one reason being that the disorganized type was defined as an altered pattern of staining with cytoplasmic expression or variable staining with some areas preserved and other areas reduced, and many cases were included in the disorganized type. We considered E-cadherin and β-catenin expression levels to be reduced when <70% of the tumor cells were stained, because the distribution of ratio of staining cells showed bipolarity.

Our data show that in 138 cases of the reduced expression of E-cadherin, 70 (51%) were the reduced expression of β-catenin, with a statistically significant correlation (P < 0.0001), and these data were consistent with reports on colon cancers by Takayama et al. (39) and esophageal cancer by Krishnadath et al. (19). Therefore, various carcinomas showed a significant correlation with expression of E-cadherin and β-catenin.

We found a significant correlation between E-cadherin expression and lymph node metastasis, especially in cases of squamous cell carcinoma. Consistent with reported data (33, 34, 36), our data show that reduced E-cadherin correlates with lymph node metastasis. When Bongiorno et al. (17) examined E-cadherin expression in 52 lung carcinomas, all metastatic cells in lymph nodes exhibited intense E-cadherin expression levels equal to and often greater than in the primary tumor (17). Therefore, reduced E-cadherin expression weakens cell-to-cell attachment, and tumor cells detach from the primary tumor, invade vessels, and migrate to lymph nodes. Once tumor cells reattach to lymph nodes, E-cadherin is strongly expressed, and lymph nodes are subject to metastases. In our study, although the E-cadherin expression did not correlate lymphatic invasion, the rate of vascular invasion was statistically high in cases with the reduced expression of E-cadherin. However, only 20 of 118 cases with reduced expression of E-cadherin showed vascular invasion, and only 11 of 118 cases showed lymphatic invasion. Because the number of cases with vascular invasion or lymphatic invasion was small, it is difficult to discuss the relationship between lymph node metastasis and vascular/lymphatic invasion.

Our findings revealed that reduction of E-cadherin is associated with the degree of differentiation. Bohn et al. (34) found a correlation between differentiation and E-cadherin expression in lung squamous cell carcinoma, and Bongiorno et al. (17) found that well-differentiated lung cancers express E-cadherin, in a preserved fashion, and that poorly differentiated tumors exhibited a reduced or disorganized staining pattern (17). Sulzer et al. (33) also found that E-cadherin expression significantly correlated with increasing tumor differentiation. In general, undifferentiated or poorly differentiated cancer cells tend to have a strong potential to invade tissues. These results suggest that reduction of E-cadherin correlates with tumor invasion.

We found that the expression of reduced β-catenin significantly correlated with a poor prognosis. Retera et al. (37) studied 101 patients with NSCLC and found that the level of β-catenin expression was a statistically significant prognostic factor. They classified the results into three categories according to the proportion of tumor cells with immunoreactivity for β-catenin on the one hand and the mean staining intensity of positively stained tumor cells on the other. Total immunostaining score was divided into low, moderate, and high scores. The mean survival time was 24.9 months for patients with a low score, 42.8 months in case of a moderate score, and 44.1 months for a high score. This is apparently the only report on the correlation between reduction of β-catenin expression with the prognosis of patients with lung cancer. In comparison with their study, we studied 331 cases of NSCLC and found a correlation between prognosis and β-catenin expression, histologically, and also that the reduced expression of β-catenin in adenocarcinoma meant a significantly unfavorable prognosis compared with

<table>
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<th>Factor</th>
<th>Relative risk (95% confidence interval)</th>
<th>P</th>
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<tbody>
<tr>
<td>Lymph node metastasis (+ vs. −)</td>
<td>4.5920 (2.7193–7.7540)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tumor stage (T1 vs. T2, T3, T4)</td>
<td>1.8677 (1.4981–2.3284)</td>
<td>&lt;0.0001</td>
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<tr>
<td>β-Catenin (preserved vs. reduced)</td>
<td>0.4520 (0.2820–0.7245)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Differentiation (well, moderate vs. poorly)</td>
<td>1.4769 (1.0843–2.0119)</td>
<td>0.0135</td>
</tr>
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</table>
cases of preserved expression. Concerning adenocarcinoma of the lung, this seems to be the first report that reduced β-catenin correlates with an unfavorable prognosis.

Shibanuma et al. (36), who examined the relationship between the expression of E-cadherin, α-β-γ-catenin, and clinicopathological factors in 81 cases of NSCLC, found statistically significant relationships between the expression of E-cadherin and lymph node metastasis and between the expression of E-cadherin and pathological stage. Dividing the 81 cases into an E-cadherin functional group and other groups, there was a statistically significant relationship between E-cadherin function and all of the clinicopathological factors (local tumor invasion, $P = 0.033$; lymph node metastasis, $P < 0.001$; pathological stage, $P < 0.001$; Ref. 36). In our study, although the correlation was not statistically significant between the E-cadherin expression and prognosis, there was weak relationship ($P = 0.0652$). When we analyzed the prognosis for patients with a combination of the expression of E-cadherin and β-catenin, cases of the reduced expression of both E-cadherin and β-catenin showed a significant unfavorable prognosis compared with either reduced expression of E-cadherin and β-catenin and that with preserved expression of both of E-cadherin and β-catenin. Because of the direct binding of E-cadherin to the β-catenin molecule, the reduced expression of both E-cadherin and β-catenin resulted in a weaker cell-cell adhesion after detachment of cancer cells from the primary lesion. Therefore, combined analysis of the expression of E-cadherin and β-catenin may be more pertinent to estimate the prognosis in NSCLC.

Hypermethylation around the promoter may be a mechanism of E-cadherin inactivation in human carcinomas, and treatment of E-cadherin-inactivated cells with a demethylating agent may lead to gene expression reversion and epithelial morphogenesis with acquisition of the homophilic cell-cell adhesive property (40). Kanai et al. (41) showed that CpG methylation around the promoter region of the E-cadherin gene correlated significantly with reduced E-cadherin expression in hepatocellular carcinoma. Sato et al. (42) reported that hypermethylation was observed in the H-cadherin gene in 9 of 20 primary lung cancers. E-cadherin expression level was not uniform, and hypermethylation around the promoter region of the E-cadherin gene may lead to the loss of E-cadherin expression.

β-Catenin is involved not only in the cadherin cell adhesion system but also in the growth signal pathway. Signals generated by Wingless/Wnt, an essential embryonal organization, induce protein expression of β-catenin. The role of β-catenin downstream of Wnt seemingly differs from that in cadherin-mediated cell adhesion, because this β-catenin, induced by Wnt, exists in the cytoplasm without binding to cadherin (11). Moreover, the overexpression of truncated β-catenin, which cannot bind with α-catenin, has an effect in embryogenesis similar to that of the wild-type of β-catenin or growth signal of Wnt (43). Wnt also functions as an oncogene in human mammary carcinogenesis (44). We found β-catenin protein expressed in the cytoplasm. However, this type of expression of β-catenin was minimal, and little influence was noted regarding clinical features and outcome in NSCLC.

It has been demonstrated that β-catenin binds with the APC tumor-supresser gene product in the cytoplasm and this complex does not include cadherin (45). The wild-type APC has little effect on β-catenin binding with cadherin, but it does decrease protein content of the cytoplasmic free β-catenin (46). The function of APC as a tumor-supresser gene might bind and limit cytoplasmic-free β-catenin. β-catenin in this status binds to T-cell factor and lymphoid enhancer factor transcription factors, which play key roles downstream of the Wingless signals (14, 15, 47). Colorectal tumors with intact APC genes were found to contain activating mutations of β-catenin that altered functionally significant phosphorylation sites (14). In 122 cases of reduced expression, $34$ (28%) showed cytoplasmic expression of the β-catenin, and the other $88$ (72%) showed noncytoplasmic expression. There was no significant correlation between the cytoplasmic expression and various clinicopathological factors. The 5-year survival rate was 52.1% for patients with cytoplasmic expression and 40.6% for those with noncytoplasmic expression, with no statistical difference ($P = 0.7822$; data not shown). In our data, lung cancer with the cytoplasmic expression of β-catenin is not as significant, as seen in cases of colon cancer.

In conclusion, the cadherin-catenin complex may have a major role in cell-cell adhesion systems, and down-regulation of E-cadherin and β-catenin indicates an unfavorable prognosis. This is a useful prognostic factor in NSCLC with β-catenin expression. For patients with a reduced expression of the cadherin-catenin complex, follow-up should be close because optional chemotherapy or radiation may be required.

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